

Prognostic importance of plasma heparin binding protein in the diagnosis of acute appendicitis

Heparin binding protein in the diagnosis acute appendicitis

Ayetullah Temiz¹, Konca Altınkaynak², Yavuz Albayrak³, Yılmaz Özdemir⁴, Saime Özbek Şebin⁵, Ceren Şen Tanırkulu⁶

¹Department of General Surgery, Erzurum Regional Training and Research Hospital, Erzurum,

²Department of Biochemistry, Erzurum Regional Training and Research Hospital, Erzurum,

³Department of General Surgery, Atatürk University School of Medicine, Erzurum,

⁴Department of Gastroenterology Surgery, Erzurum Regional Training and Research Hospital, Erzurum,

⁵Department of Physiology, Atatürk University School of Medicine, Erzurum,

⁶Department of Emergency Medicine, Konya Education and Research Hospital, Konya, Turkey

Abstract

Aim: In recent years, it has been found that plasma levels of Heparin Binding Protein (HBP) are elevated in systemic inflammatory conditions. In this study, we aimed to demonstrate the diagnostic value of HBP in patients with acute appendicitis (AA). **Material and Method:** Sixty patients with acute appendicitis and 27 healthy subjects were included in the study. Plasma HBP levels were measured in the patient groups and control groups and the results were compared.

Results: The average HBP level in the 60 patients with acute appendicitis (AA) was 9.63 ± 3.64 ng/ml, while the average HBP value of the healthy group was 7.65 ± 2.52 ng/ml. When the groups were compared, the HBP serum levels were significantly lower ($p = 0.02$) in the healthy control group. The area under the curve (AUC) was 0.785 in the ROC curve for diagnosis of acute appendicitis using HBP. **Discussion:** This study has significance as the first study to investigate the HBP level in patients with acute appendicitis, and HBP may be a potential new marker for the diagnosis of acute appendicitis.

Keywords

Acute Appendicitis; Heparin Binding Protein; Inflammation

DOI:10.4328/ACAM.6004 Received: 16.08.2018 Accepted: 25.09.2018 Published Online: 26.09.2018 Printed: 01.07.2019 Ann Clin Anal Med 2019;10(4): 491-5
Corresponding Author: Ayetullah Temiz, Department of General Surgery, Erzurum Regional Training and Research Hospital, 25100 Erzurum, Turkey.
T.: +90 4422325248 E-Mail: temiz-49@hotmail.com
ORCID ID: <https://orcid.org/0000-0003-2178-3369>

Introduction

Acute appendicitis (AA) is the most frequent cause of acute abdominal pain with a lifetime risk of 7%, representing an important proportion of emergency surgical procedures. In the US, approximately 300 000 people undergo appendectomies annually in acute care hospitals [1,2]. An AA diagnosis is usually based on a combination of clinical information including symptoms (e.g. right lower quadrant abdominal pain, nausea and/or vomiting, high fever (38°C), abdominal rigidity and painful urination), physical examination findings, traditional biomarkers such as white blood cell count (WBC), mean platelet volume (MPV), absolute neutrophil count and C-reactive protein (CRP) and radiographic imaging (e.g. ultrasound and computed tomography scans) [3-5]. Clinical diagnosis of AA is often difficult even for experienced surgeons, and the rate of negative explorations can reach 20%–30% [6-7]. Delays in the diagnosis of AA may lead to perforation and the development of other complications. Therefore, there is a need for new laboratory methods that are both easy and inexpensive to perform for the diagnosis of AA.

Heparin-binding protein (HBP), also known as azurocidin or CAP37, is stored in secretory vesicles and azurophilic granules of neutrophils and is released early upon neutrophil adhesion and during neutrophil extravasation. Bacterial products induce the release of HBP leading to increased vascular leakage by acting on endothelial cells through unknown mechanisms [8]. In clinical investigations, the release of HBP has been demonstrated to be caused by a wide array of bacteria in various infectious diseases [9-11]. However, a search of the medical literature did not find a study about the place of HBP as an inflammatory marker in patients with AA. In this study, we aim to demonstrate the diagnostic value of HBP in patients with AA.

Material and Method

Study Participants:

This study was carried out at the General Surgery Clinic of Erzurum Regional Education and Research Hospital between September 2016 and November 2016 in 60 patients who were diagnosed with acute appendicitis and in 27 healthy control individuals. The present study was approved by the Erzurum Regional Training and Research Hospital Institutional Review Board (approval number: 2015/10-105). A written informed consent from the patient was waived because the present study was performed prospectively.

The healthy control group was selected from healthy persons who came to the hospital without any complaints, just for purposes of a routine checkup in the Infection Diseases Clinic, and who did not conform to the exclusion criteria.

Inclusion and Exclusion Criteria:

All of the patients underwent operations for appendicitis based on their history, physical findings, and relevant clinical data. Postoperatively, the removed appendix was sent for histopathological examination. Cases, where the histopathology was not consistent with appendicitis, were excluded from the study. The exclusion criteria for entry into the study were heart failure, peripheral vascular disease, hematological disorders, acute or chronic infection, cancer, prior antibiotic therapy, age < 10

years, pregnancy, hepatic diseases, and other known inflammatory conditions. None of the patients had received prior anticoagulant medications, nonsteroidal anti-inflammatory drugs or oral contraceptives.

Biochemical Analysis:

Venous blood samples were obtained from the patient group and the control group and were collected into hemogram test tubes containing EDTA. The samples were centrifuged at 4000 rpm for 10 minutes, and then, they were stored at –80°C until the day the analysis was performed. Plasma levels of HBP were measured by a Human azurocidin ELISA kit (Bioassay Technology Laboratory Cat. China) using a microplate reader (Bio-Tek Power Wave XS).

Statistical Analysis:

All statistical analyses of the data were performed with the Statistical Package for Social Sciences (SPSS) 16.0 for Windows (SPSS Inc. Chicago, IL, USA). Data distribution was evaluated using the Kolmogorov–Smirnov test. Continuous variables are expressed as mean ± standard derivations (SD) and categorical variables as frequencies (percentages). The significance of each difference between continuous variables was explored with the Mann-Whitney U-test. The significance of each difference between categorical variables was compared using Pearson's Chi-squared test. Receiver operating characteristic (ROC) curve analysis was used to define the optimal cut-offs of the WBC, CRP, and HBP, for which specificities, sensitivities, positive and negative predictive values and overall accuracies, were calculated. A p-value <0.05 was considered to reflect statistical significance.

Results

Of the 60 patients who underwent appendectomies, 36 were males, and 24 were females, and of the healthy group, 12 were males, and 15 were females. Demographic and clinical characteristics concerning age, gender distribution, WBC, CRP, and HBP levels are presented in Table 1.

Table 1. Comparison of groups according to age, gender and laboratory data

	Acute appendicitis	Control
Age	34,45±13,40	32,00±8,71
Gender (M/F)	36/24	12/15
White Blood Cell (x109 /L)	14400±4670	7430±1140
C-Reaktif Protein (ng/ml)	3,91±5,77	0,33±0,03
Heparin Binding Protein(ng/ml)	9,63±3,64	7,65±252

The HBP, WBC, and CRP results for patients and control groups are shown in Figure 1. The average HBP level in the 60 patients with AA was 9.63 ± 3.64 ng/ml, while the average HBP value of the healthy group was 7.65 ± 2.52 ng/ml. When the groups were compared, the HBP serum levels were significantly lower (p = 0.02) in the healthy control group (Figure 1). The average WBC level in the 60 patients with AA was 14400 ± 4670 ng/ml, while the average WBC value in the healthy group was 7.43 ± 1.14 ng/ml. When the groups were compared, the WBC serum levels were significantly lower (p < 0.001) in the healthy control group

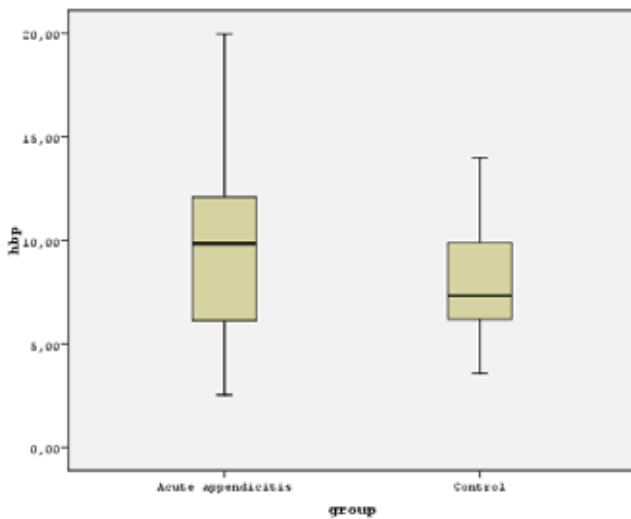


Figure 1. Heparin Binding protein counts by category of groups. (HBP ng/ml)

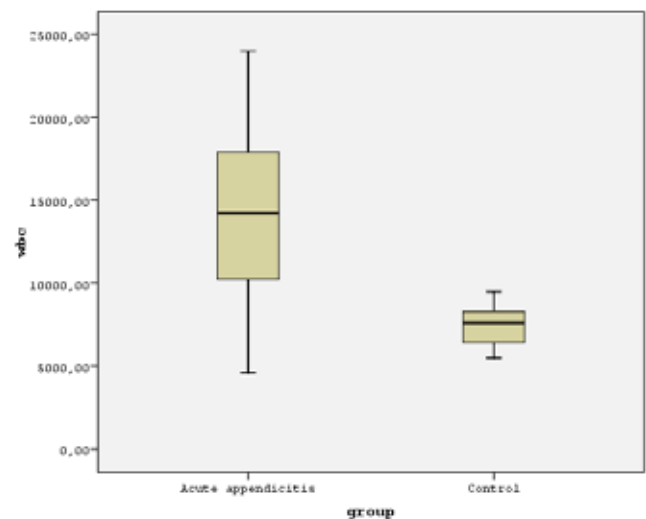


Figure 2. Absolute white blood cell (WBC) counts by category of groups. (WBC X 10⁶/L)

(Figure 2). The average CRP level in the 60 patients with AA was 3.91 ± 5.77 ng/ml, while the average CRP value in the healthy group was 0.33 ± 0.03 ng/ml. When the groups were compared, the CRP serum levels were significantly lower ($p < 0.001$) in the healthy control (Figure 3). In the ROC curve analysis, sensitivity rates for HBP, WBC, and CRP rate were 86.7%, 64.4%, 66.7%, respectively, while specificity rates were 71.4%, 76.2% and 76.2%, respectively (Figure 4) (Table 2). In addition, the distribution of HBP levels in patients and in the control group is shown in Table 2. For HBP, the area under the curve (AUC) was 0.785 in the ROC curve for the diagnosis of acute appendicitis.

Discussion

The aim of this study is to evaluate the diagnostic value of plasma HBP levels in patients with suspected AA. Acute appendicitis (AA) is one of the most commonly encountered emergency presentations to general surgical services and requires emergency surgical intervention [12]. In the pathogenesis of acute appendicitis, bacterial invasion of the appendicular wall occurs, and the consequent inflammatory reaction leads to purulent infection. In many cases, appendices are surgically removed due to inadequate diagnostic laboratory evidence. Unnecessary removal of the appendix due to a lack of reliable laboratory data has not only become a burden on healthcare systems and increased suffering in patients but also it has reduced quality of life in patients [13]. For this reason, the need is great for new, easily applied and inexpensive diagnostic tools that have high diagnostic value for AA and little operator dependence. HBP is included in azurophilic granules and secretory vesicles of neutrophils and is released upon fusion of azurophilic granules and secretory vesicles with the plasma membrane. The basal release of HBP is augmented upon $\beta 2$ integrin-dependent adhesion of neutrophils to the vascular endothelium during inflammation [14,15]. At the site of infection, it is also secreted from azurophil granules during phagocytosis, where it exhibits antimicrobial activity and is responsible for the recruitment and activation of monocytes and other inflammatory mediators. It is also internalised by monocytes to prolong survival and enhance cytokine production [16]. Therefore, HBP directly contrib-

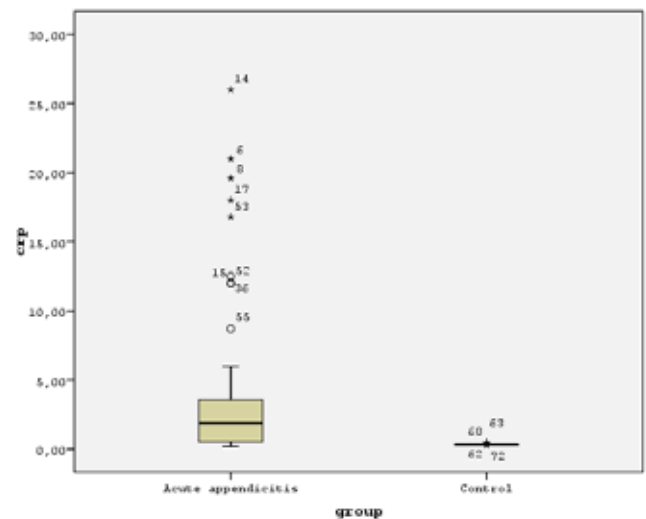


Figure 3. C-Reaktif Protein counts by category of groups. (mg/dl)

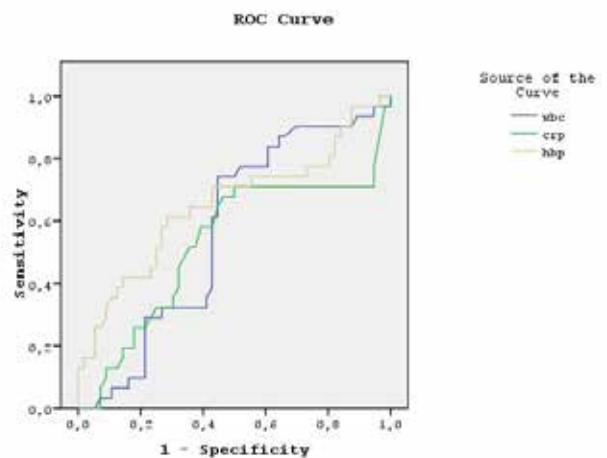


Figure 4. ROC curve used to distinguish AA patients from controls

utes to the maintenance and progression of inflammation [17]. In a recent study, increased HBP was shown to be associated with increased permeability and ARDS in human sepsis. In addition, unfractionated heparin and low molecular weight heparins are potential drugs to prevent excessive HBP-induced increases

Table 2. Effectiveness of the parameters in the diagnosis of acute appendicitis

	Sensitivity	Specificity	PPV	NPV	CUT-OFF	AUC
White blood cell	64.4%	76.2%	74.4%	66.7%	9500	0.656
C-reaktif protein	66.7%	76.2%	75.0%	68.1%	0,49	0,667
Heparin binding protein	86.7%	71.4%	76.5%	83.3%	9.75	0.785

NPV: Negative predictive value; PPD: Positive predictive value.

in vascular leak in sepsis [18]. Linder et al. [11] reported that plasma HBP levels in patients with septic shock or sepsis were significantly higher than those in non-septic patients in the intensive care unit, and HBP correlated with severity of illness. It has also been observed that elevated HBP during admission to the hospital is associated with increased risk of death. Therefore, it is suggested that repeated HBP measurement in the intensive care unit can help to monitor treatment and predict the outcome in patients with severe infections. In another study in intensive care patients, it was shown that a high concentration of HBP in plasma on admission to the intensive care unit is associated with not only respiratory and circulatory failure later during the intensive care unit care period, but also with increased 30-day mortality [19].

In a study of patients with urinary tract infections, urinary HBP levels were found to be higher in patients with urinary tract infection compared with healthy subjects [20]. In a similar study, plasma HBP levels were found to be significantly higher in patients with pediatric acute pyelonephritis [21]. In a recent study, it was shown that plasma HBP levels in acute lung injury (ALI) / acute respiratory distress syndrome (ARDS) patients were significantly higher compared with cardiogenic pulmonary edema patients, and they have suggested that this is a strong indicator for short-term mortality in ALI/ARDS patients. It also suggests that HBP levels may both guide clinical management and shed light on appropriate treatment in this group of patients [22].

In a study on central nervous system infections, it was shown that HBP levels in the Cerebrospinal fluid in patients with acute bacterial meningitis increased significantly compared with other central nervous system infected patients [10]. HBP levels also increased significantly in inflammatory diseases caused by *Leishmania chagasi*, influenza (H1N1) virus and *Leptospira interrogans* [23,24,25].

In our study, HBP levels were significantly higher in patients with acute appendicitis compared with the control group. We have also found that HBP is more sensitive than WBC and CRP in confirming the disease in the ROC curve for both sensitivity and specificity in the diagnosis of acute appendicitis. In addition, in the AA group, the value of the AUC was as high as 0.785. For this reason, the utility of HBP in diagnosing acute appendicitis is considered high.

Conclusion

Worldwide, AA is one of the most common surgical pathologies. When the correct diagnosis is not made, both complications, such as perforation, and negative surgical procedures can occur. Additional laboratory tests are needed to confirm the diagnosis to reduce these complications and negative appendectomies. This study has significance as the first study to investigate HBP levels in patients with acute appendicitis, and to suggest that HBP may be a potential new marker for the

diagnosis of AA. Therefore, HBP can be used as a diagnostic marker for AA because HBP levels are significantly higher in patients with AA.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:

Temiz A, Altınkaynak K, Albayrak Y, Özdemir Y, Şebin SÖ, Tanrıku CŞ. Prognostic importance of plasma heparin binding protein in the diagnosis of acute appendicitis. *Ann Clin Anal Med* 2019;10(4): 491-5.